REMARKS

Status of the Claims

Claims 1-19 and 31-39 are under examination. Claims 20-30 were cancelled.

Applicants acknowledge with appreciation the Examiner's withdrawal of the rejections made in the previous office action, including the rejection of claim 1 under 35 U.S.C. 102(b) as being anticipated by Hodgson (US 2002/0025561) and the rejections of claims 1-19 and 31-39 under 35 U.S.C. 103(a) over Hodgson, in view of Slater et al. (US 2005/0074883), Gokhale et al. (*Science*, 1999, 284:482-485), and Santi et al. (US 2004/0166567).

However, claims 1-19 and 31-39 are rejected by the Examiner as allegedly obvious over Hodgson (US 2002/0025561), in view of Padgett et al. (*Gene*, 1996, 168:31-35), Resnick et al. (WO 98/01573), certain "Steward et al.," and Gokhale et al. (*Science*, 1999, 284:482-485) under 35 U.S.C. § 103(a).

In this paper, claims 2, 6, 14, 19, 32, 36, 37, and 39 have been amended to correct informalities. In particular, claims 2 and 14 are amended by inserting the missing term "are"; claim 36 is amended by inserting the missing term "claim" and correcting the improper punctuation; claim 35 is also amended by inserting the missing term "and" and correcting the improper punctuations; claim 19 is amended to make it more clearly dependent from claim 14; and claims 6, 32, 37, and 39 are corrected to recite the proper Markush group language. Thus, the amendments do not introduce any new matter.

Reconsideration is respectfully requested in view of the following remarks.

The Invention

The instant invention is drawn, in one aspect, to a method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising: a) providing at least three DNA molecules, each comprising a DNA segment and a vector segment, wherein each DNA segment is adjacent to one or two other DNA segments in the ligation product, wherein each DNA segment comprises a first region having sequence identity with a first adjacent DNA segment and a second region having sequence identity with a second adjacent DNA segment, if present, and wherein each vector segment comprises a selectable marker and/or a counter-selectable

marker; b) cleaving each DNA vector to produce a DNA segment with one or two ligatable ends, each said ligatable end comprising at least a portion of the region having sequence identity with an adjacent DNA segment; wherein at least one segment comprises two such ligatable ends after cleavage and wherein at least two DNA segments comprise exactly one such ligatable end, c) simultaneously ligating each DNA segment to the adjacent DNA segment or segments, wherein the DNA segment with one or two ligatable ends is not purified prior to said ligating; and d) selecting a ligation product comprising sequences from each of said DNA segments in a predetermined order, wherein said selection is based on the presence in a vector comprising the ligation product of a selectable marker and counter selectable marker of at least one of said three DNA vectors.

The invention described allows rapid, economic and efficient preparation of artificial genes. Unlike the prior art methods described by the Examiner, the invention provides a practical method for making very large synthetic genes (e.g., including genes greater than 20 kbp in length) by a process including simultaneous ligation of multiple fragments. The claimed method makes use of a combination of three different types of vectors, and, in contrast to prior art methods, does not require multiple rounds of purification of short isolated inserts (e.g., by gel electrophoresis). Elimination of the need for isolation and recloning of small polynucleotide fragments is a significant advantage over the prior art methods in the references cited by the Examiner.

The Rejection of Claims under 35 U.S.C. § 103(a)

The Examiner rejected claims 1-19 and 31-39 as allegedly obvious over Hodgson (US 2002/0025561) (hereinafter "Hodgson"), in view of Padgett et al. (Gene, 1996, 168:31-35.) (hereinafter "Padgett et al."), Resnick et al. (WO 98/01573) (hereinafter "Resnick et al."), "Stewart et al.," and Gokhale et al. (*Science*, 1999, 284:482-485) (hereinafter "Gokhale et al."). In the Office Action, although the Examiner cited certain "Steward et al." along with Hodgson, Padgett et al., Resnick et al., and Gokhale et al., the citation of Steward et al. was not provided; therefore, Applicants will only be able to address whether the Examiner established prima facie obviousness of the present invention over Hodgson in view of Padgett et al., Resnick et al., and Gokhale et al.

Although the Examiner used the sweeping language to reject all the pending claims, i.e., claims 1-19 and 31-39, the Examiner provided reasons only in a haphazard manner by

sporadically pointing out certain limitations of the claims allegedly found in the references cited. The Examiner has failed to provide enough evidence to show that all the limitations of the rejected claims have been taught by the references cited.

To establish prima facie obviousness, all the limitations of a claim must be taught by the references. In making a rejection, the Examiner should not engage a hindsight picking and choosing, because retrospectively, most, if not all, significant scientific or technological advancements may appear obvious, but in fact highly innovative given the state of art in which the advancements are made. Every scientific or technological advancement is built upon the existing knowledge and technology. Moreover, to establish obviousness, it is not enough that the pieces of information about the elements of a claim could be found in the references, but it requires that the information combined would teach all aspects of an invention sought to be patented and render the invention obvious.

In this case, the Examiner has picked out various pieces of information from the references cited, which combined, Applicants respectfully submit, do not teach the present invention. Moreover, as will be discussed below, the relevance of some references is questionable.

Analysis and Arguments

In this section, Applicants will analyze the references cited by the Examiner and demonstrate that the references as a whole are not sufficient to establish *prima facie* obviousness of the claims rejected. Applicants will address impropriety of the rejections made to independent claims 1, 2, 14, and 35 in turn first and then address the same for the dependent claims.

I. Claim 1

Claim 1 is recited:

> 1. A method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising:
>
> a) providing at least three DNA molecules, each comprising a DNA segment and a vector segment, wherein each DNA segment is adjacent to one or two other DNA segments in the ligation product, wherein each DNA segment comprises a first region having sequence identity with a first adjacent DNA segment and a second region having sequence identity with a second adjacent DNA segment, if present, and wherein each vector segment comprises a selectable marker and/or a counter-selectable marker;

b) cleaving each DNA vector to produce a DNA segment with one or two ligatable ends, each said ligatable end comprising at least a portion of the region having sequence identity with an adjacent DNA segment; wherein at least one segment comprises two such ligatable ends after cleavage and wherein at least two DNA segments comprise exactly one such ligatable end,

c) simultaneously ligating each DNA segment to the adjacent DNA segment or segments, wherein the DNA segment with one or two ligatable ends is not purified prior to said ligating; and

d) selecting a ligation product comprising sequences from each of said DNA segments in a predetermined order, wherein said selection is based on the presence in a vector comprising the ligation product of a selectable marker and counter selectable marker of at least one of said three DNA vectors.

A. Hodgson (US 2002/0025561)

The Examiner makes the assertion that:

> Hodgson teaches a method of obtaining a synthetic gene by ligating three DNA segments, the method comprising: (a) providing three different DNA vectors each comprising a selectable marker and a different DNA insert, wherein all DNA inserts are flanked by identical type IIS restriction sites, (b) cleaving each DNA vector with a type IIS enzyme to generate segments with region of identity (ligatable ends) with an adjacent segment, (c) simultaneously ligating the three DNA segments, and selecting the ligation product based on the presence in the vector of the selectable marker; one or more of the DNA segments could comprise the vector and the final ligation product is a complete recombinant DNA/vector, which could be made either linear or circular (i.e., the final ligation product comprises a selection marker from one of the three vectors), and (d) transforming cells with the final ligation product and selecting the transformants comprising the ligation product based on the presence of the selection marker above (claim 1) (p. 3, paragraphs 0030 and 0031, claims 1, 5, 9, and 10).

(Office Action, pages 3-4). A close reading of the text in Hodgson as the Examiner purportedly directed, i.e., paragraphs 0030 and 0031 and claims 1, 5, 9, and 10, does not reveal teaching of at least the following elements: (a) "*three different DNA vectors each comprising a selectable marker and a different DNA insert*"; (c) "simultaneously ligating the three DNA segments, and *selecting the ligation product based on the presence in the vector of the selectable marker*"; and (d) "transforming cells with the final ligation product and *selecting the transformants comprising the ligation product based on the presence of the selection marker*." In contrast, Hodgson teaches: (a) preparing a series of overlapping DNA molecules; (b) *cloning the DNA molecules into a vector*; (c) *validating the insert fragments*; (d) digesting the clones; (e) *purifying the insert*

fragments away from the vector fragments; (f) annealing and ligating the insert fragments together; and (g) characterizing the resulting DNA construct. (Hodgson, claim 1).

As discussed in the response to the previous office action, Hodgson does not disclose or suggest various limitations of method claim 1: (1) that the fragments are ligated without an additional step of purification between the step of cleaving those fragments from vectors and the step of ligating these fragments; and (2) that each vector segment comprises a selectable marker and/or a counter-selectable marker. First, Hodgson explicitly states that "the released insert fragments are isolated and purified from the vector fragments. (for example, by isolation from an agarose gel, or by ion-pair reverse-phase high performance liquid chromatography [i.e., the WAVE machine, Transgenomic, Inc., Omaha, Nebr.])." (Hodgson, paragraph [0030], emphasis added; *see also* Hodgson, paragraph [0051] ("This [insert isolation and purification] can be done by any methods but is usually done by digesting clones with Sap 1, and by running the digested DNA on agarose gel.... Gel plugs containing the bands ... are removed from the gel [and] (sic) extracted using QIAquick gel extraction kits.")).

In yet another preferred embodiment, the insert DNA fragments are created from synthetic oligodeoxynucleotides (ODNs), by means of any methods for using either annealed, double-stranded ODNs, or DNA molecules made by polymerizing annealed ODN sequences to fill gaps. Synthetic ODNs can be ordered from a commercial supplier (e.g., MWG, Inc., Charlotte, N.C.), and should be purified by a fastidious process, such as by MWG's standard, high-purity salt-free (HPSF) process, prior to cloning. (Hodgson, paragraph [0040]).

It is a well-settled law that an "omission of an element and retention of its function is an indicia of unobviousness." *In re Edge*, 359 F2d 892, 149 USPQ 556 (CCPA 1966)." MPEP § 2144.04.II.B.

Second, a fundamental aspect of the invention is the coordinated use of structurally distinct vectors comprising selectable and/or counter selectable markers. In contrast, as the Examiner has acknowledged: "Hodgson and Padgett et al. do not [t]each (sic) vector as comprising a selectable and counter selectable marker, with each vector comprising a distinct set of selectable and counter selectable marker (claims 2-10, 13-19 and 31-39)." (See Office Action, page 5, second paragraph). Hodgson does disclose that the colonies are selected using blue-white selection indicative of functional β-gal expression or absence thereof. This simplified clone selection method is most suitable, if not singularly suitable, for methods entailing the

presence of only one vector. Notably, the blue-white selection method does not allow differentiation between the clones having the desired construct and the clones having a single insert ligated back into the vector it was excised from. Therefore, in a sense, Hodgson teaches away from the subject matter of the independent claims 1, 2, and 35.

In addition, since Hodgson purifies his fragments of interest, one of skill in the art would have figured out that counter selection markers are not needed in Hodgson's invention. As provided in the Declaration of Dr. Reisinger submitted in response to the previous office action, the use of two-marker vectors provided unexpected and clear advantage over one-marker vectors. Specifically, when the inventors used one-marker vectors, they found that 28% of the clones were false-positives. Such a high percentage of false positive clones necessitates additional steps of verification whether the selected clone is truly or falsely positive. In contrast, when two-marker vectors were used, no false positive clones were encountered. Accordingly, the use of two-marker vectors, as claimed by the applicants, makes the multiple-fragment cloning procedure much more efficient.

Hodgson admits that cloning using Type IIS enzymes is a paradigm of trial and error, and that more work needs to be done before cloning multi-segment DNA molecules. (*See* Hodgson, paragraph [0009]). "Although a number of investigators successfully used class IIS enzymes for cloning, the construction of complex DNA molecules by this approach was limited to a small number of examples." (Hodgson, paragraph [0009], emphasis added). It follows that at the time the instant invention was made, there was no reasonable expectation of success in cloning multiple pieces of DNA into one vector through a one-step process.

B. Padgett et al. (*Gene*, 1996, 168:31-35)

The Examiner acknowledged that "Hodgson does not teach ligating the desired DNA segments without purifying the digested fragments," but asserted that "at the time the invention was made, ligating digested fragments without purifying them was taught by the prior art; the prior art also taught that the presence of the type IIS restriction endonuclease in the ligation reaction reduces the cloning time and provides selection for the desired ligation product." (Office Action, page 5, top, referring to Padgett et al., p. 34, column 1 and p. 35, column 1). In fact, on page 34, column 1, Padgett et al. does not mention, let alone "teach," that ligating digested fragments does not need purifying them.

Padgett et al. described a method for cloning a DNA sequence into a desired location without the limitation of naturally occurring restriction sites. The technique employs the polymerase chain reaction (PCR) combined with the capacity of the type IIS restriction endonuclease (Enase) Eam1104I to cut outside its recognition sequence. For at least several reasons as discussed below, Padgett et al. cannot serve the purpose as the Examiner has intended it to.

First, regarding the Examiner's point that Padgett et al. teach that they observed increased efficiency using their non-gel isolation procedure, Padgett et al. is irrelevant to the present invention because the increased efficiency required the use of a methyl-sensitive Type IIS restriction enzyme and methylated CTP, which is inapposite to the present invention. Including the methylated CTP in the PCR reaction acted to inhibit digestion of Eam1104I sites within the amplicon, and thus resulted in increased cloning efficiencies by ensuring that only the non-methylated, Eam1104I site on the 5' primer, and not the Eam1104I site within the amplicon, was competent for digestion. Quite simply, no increase in cloning efficiency would have been observed by Padgett et al. without the use of a methyl-sensitive Type IIs restriction enzyme and methylated CTP.

Second, Padgett only teaches simple cloning of PCR fragments with a single vector, which does not approach the complexity of cloning 3 or 4 vectors simultaneously as with the present invention.

Third, absent the Examiner's elaboration, Applicants venture to speculate that the Examiner might have referred the "teaching" to the sentence: "The PCR products were digested with Eam1104I and subsequently ligated together in the presence of ENase." However, Applicants respectfully submit that by merely mentioning the order of the two operations, i.e., digestion and ligation, Padgett et al. is short of teaching "ligating digested fragments without purifying them." As for page 35, column 1 of Padgett et al. the Examiner referred to, Applicants also can only speculate that the Examiner considered the sentence "[c]oncurrent digestion of insert and target PCR products and a brief ligation step of the digested material in the presence of the ENase further reduce the overall cloning time" as teaching "ligating digested fragments without purifying them." Again, Applicants respectfully disagree. Given that Padgett et al. does not teach "ligating digested fragments without purifying them" in the experimental or discussion section, the mere mentioning of "[c]oncurrent digestion of insert and target PCR products and a

brief ligation step of the digested mater" would add little, if anything, to the teaching. The mere language would not teach the concept of "ligating digested fragments without purifying them," because between the step of "concurrent digestion of insertion and target PCR products" and the "a brief ligation step" may well include a purification step.

Simply put, the fact that certain step is not mentioned does not necessarily indicate that this step is omitted. Thus, to interpret the language to mean the step of "digestion of insert and target PCR products" and the step of "ligation" are "concurrent" would be not only implausible but also contrary to experimental conventions.

Fourth, even assuming that Padgett et al. does not involve an isolation or purification step, it still would not be applicable to the present case. In the present invention, vectors contain inserts and upon digesting vectors and re-ligation of inserts, unnecessary vectors are discarded. Thus, the ratio of inserts to the respective vectors is 1:1. Whereas in Padgett et al., inserts are amplified by means of PCR. Thus, in the digestion (and ligation) mixtures, the vectors and the respective inserts are present in a different ratio. Assuming that Padgett performed 25 cycles of PCR with 100% efficiency of PCR amplification, the ratio of inserts to vectors would be about 16,000,000 (2^{25}) to 1, and assuming a 40% efficiency, the ratio of inserts to vectors would be about 6000 to 1. Even assuming a 20% efficiency of PCR amplification, the ratio of inserts to their respective vectors would still be more than 100 to 1. Thus, for all intents and purposes, in a practical sense, the PCR mix of Padgett et al. has been purified. Thus, without explicitly stating so, Padgett et al. does teach purification of the insert by enriching the ratio of insert to vector through PCR.

If one considers the teachings of Hodgson and Padgett as a whole, these teachings may be reconciled as follows: if one uses PCR to enrich the relative amount of the inserts or if the inserts are provided as vectorless oligonucleotides, then purification may be unnecessary (according to Padgett) but if the inserts are excised from their respective vectors and not enriched (by PCR or otherwise), then they must be purified (according to Hodgson). Notably, the experimental mixture recited in the claim is more similar to the experimental design described in Hodgson than to Padgett. Accordingly, the teaching of Hodgson (purification) is more relevant than the teaching of Padgett (no purification if insert amounts are enriched).

Moreover, notably, Hodgson was filed after Padgett et al. was published. Thus, Padgett et al. does not challenge the disclosure that "the construction of complex DNA molecules by this

approach was limited to a small number of examples." Further, it may be said that the state of the art was more advanced at the time of Hodgson publication, compared to the state of the art at the time of Padgett publication. And yet, at the later time, the state of the art leads to a conclusion that the construction of complex DNA molecules is generally unsuccessful. Accordingly, the reasonable expectation of success is still lacking, even if the Examiner's assertion were fully accredited.

Therefore, Padgett et al. does not remedy Hodgson in respect of a skipping isolation/purification step, as the Examiner had attempted to show.

C. Resnick et al. (WO 98/01573)

The Examiner cited Resnick et al. purportedly to show the teaching of use selectable and counter selectable markers. The Examiner asserted that "at the time the invention was made, the prior art taught the use of a combination of vectors each vector having a distinct set of selectable and counter selectable markers for the accurate selection of the final recombinant product comprising the desired insert, wherein the selectable marker could be the tetracycline resistance gene and the counter selectable marker could b[e] (sic) the ccdB or SacB gene." (Office Action, page 5, bottom paragraph.) Applicants respectfully submit that Resnick et al. is not applicable to the present case due to the different contexts presented by the two cases.

Resnick et al. is directed to a method of making a yeast artificial chromosome (YAC) comprising introducing into a yeast cells a population of nucleic acids and a vector, wherein the vector comprises a yeast centromere, a selectable marker, a yeast telomere, and a sequence which can recombine with a region of a nucleic acid within the population of nucleic acids, whereby *in vivo* recombination makes the YAC.

The selectable marker or counter selectable marker is used in different contexts in Resnick et al. than in the instant application. First, Resnick et al. emphasized that the selectable marker and counter selectable markers are used in a method of introducing a population of nucleic acids into yeast cells; second the method is used to create the YAC in vivo. In contrast, the present invention involves use of selectable marker and counter selectable markers to prepare precise genes encoding PKS modules, which are entirely done in vitro, and the product is used entirely for synthetic purposes. More significantly, Resnick et al. does not teach use of selectable marker and counter selectable marker in the same fashion as in the present invention.

Applicants respectfully submit that the Examiner's assertion does not constitute a faithful reading of the reference. Simply because a reference mentions use of a selectable marker or counter selectable marker, it does not necessarily lead to the conclusion that any future use of the same would be obvious regardless of the contexts in which the selectable marker or counter selectable marker is used.

Although as Resnick et al. stated, "selectable marker" and "counter selectable marker" are two terms familiar to one of ordinary skill in the art, use of them in a different context may not be feasible, or if feasible, may not be beneficial. In other words, whether the selectable marker and/or counter selectable marker would work as intended, i.e., the outcome of such uses, is not obvious. For one, Hodgson did not use them.

Moreover, a closer look at Resnick et al. would reveal that this reference is non-enabling when applied to the present invention, because the method it teaches relies solely on the use of homologous recombination to create the large YAC sequence, not a recombinant, single-tube cloning method as with the present invention. In fact, Resnick et al. teaches away from the present invention by stating that the use of recombinant cloning methods, such as the one with the present invention, is problematic (see Resnick et al., page 2, lines 28-30), which is what motivated them to use homologous recombinant methods instead.

Therefore, Applicants respectfully submit that Resnick et al. does not "teach" the use of selectable and counter selectable markers as in the present invention.

D. Gokhale et al. (*Science*, 1999, 284:482-485)

Gokhale et al. is cited by the Examiner purportedly to show motivation to make PKS genes; however, the relationship of this reference with the instant application, if it exists at al, is tenuous.

As Applicants discussed in various occasions responding to the Examiner's previous rejections, Gokhale et al. describes recombining modules from naturally occurring PKSs using conventional recombinant techniques. Gokhale et al. showed that modules that are not naturally found in the same polypeptide can be combined to produce catalytically functional products.

Gokhale et al. does not have any bearing on the concurrent, directional cloning of multiple synthons as claimed in this application, given that none of the independent claims of the instant application, namely, claims 1, 2, 14, and 35, recite a specific gene such as recombinant

PKS. In any event, if Gokhale et al. were relevant at all to the instant application, it would only be because it mentioned the PKS module, which is only remotely related to claims 11 and 12 of this application. Accordingly, Gokhale et al. is irrelevant to the analysis of obviousness of claims 1, 2, 14, and 35.

Thus, Applicants respectfully submit that citation of Gokhale et al. does not lend a support for establishing prima facie obviousness, and on the contrary, the very citation of this tenuously related reference by the Examiner indicates that the invention disclosed in this application is highly innovative, lacking pertinent motivating references, thus unobvious.

Moreover, Gokhale et al. does provide support for the fundamental need in the art at that time for cloning large DNA segments, including, but not limited to, PKSs, as evidenced by the large number of DNA manipulations outlined in the methods that were required to actually clone the PKSs tested. The present invention answers the call for the need. However, Gokhale et al. simply does not provide motivation for one to combine Hodgson and other references cited; nor would the combination of them teach the present invention.

Accordingly, Applicants respectfully request that the rejection of claim 1 under 35 U.S.C. § 103(a) over Hodgson, in view of Padgett et al., Resnick et al., certain "Stewart et al.," and Gokhale et al. be withdrawn.

II. Claim 2

Claim 2 is directed to a method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order. Claim 2 shares various attributes with claim 1 that are not disclosed by the references cited, which include, for example, (a) the use of *three types of DNA molecules, each comprising a DNA segment, a selectable marker, and a counter selectable* and (c) *without purifying the products of said cleaving of each DNA molecule*, ligating the resulting fragments to each other thereby producing a ligation product that comprises sequences from each of said DNA segments in a predetermined order. As discussed above, these unique attributes alone should overcome the references cited by the Examiner.

Moreover, reference to paragraphs [0231]-[0235], the Figures, and the remainder of the specification illustrates that Type 1, Type 2 and Type 3 vectors have other differences, not

limited to the position of the selectable marker with relation to other vector elements, for example:

> *each said Type 3 DNA molecule comprises a DNA segment, a third counter-selectable marker, a 5-prime cleavage site and a 3-prime cleavage site…*;
>
> wherein said *first, second and third counter-selectable markers are independently selected and are the same or different*;
>
> wherein said *first and third cleavage sites are the same or are compatible*;
>
> wherein said *second and fourth cleavage sites are independently selected and are the same or are different*; and,
>
> wherein *each 5-prime and 3-prime cleavage site is independently selected in each Type 3 DNA molecule and are the same or are different….*

None of these definitions are found in the references cited. Therefore, Applicants respectfully request that rejection of claim 2 under 35 U.S.C. § 103(a) be withdrawn.

III. Claim 14

Claim 14 is directed to a composition comprising an unpurified cleavage product having similar attributes to those recited in claim 2, namely (a) the use of *three types of DNA molecules, each comprising a DNA segment, a selectable marker, and a counter selectable* and (c) *without purifying the products of said cleaving of each DNA molecule*, ligating the resulting fragments to each other thereby producing a ligation product that comprises sequences from each of said DNA segments in a predetermined order. Moreover, claim 14 also defines more specifically that:

> *at least one Type 3 DNA molecule, said DNA molecule comprising a DNA segment, a third counter-selectable marker, a 5-prime cleavage site and a 3-prime cleavage site …*;
>
> wherein *said first and second selectable markers are different*;
>
> wherein *said first, second and third counter-selectable markers are independently selected and are the same or different*;
>
> wherein *said first and third cleavage sites are the same or are compatible*;
>
> wherein *said second and fourth cleavage sites are independently selected and are the same or are different*; and,
>
> wherein *each 5-prime and 3-prime cleavage site is independently selected; and a DNA ligase.*

Thus, in claim 14, the composition recited therein requires the presence of both DNA ligase and an unpurified endonuclease digestion product. As discussed above, Hodgson teaches away from using unpurified products of the digestion reaction, and one following Hodgson

would not consider adding ligase to the digestion mix before purifying the fragments of interest. For these reasons and reasons discussed above, all the references combined would not render claim 14 obvious.

Therefore, Applicants respectfully request that rejection of claim 14 under 35 U.S.C. § 103(a) be withdrawn.

IV. Claim 35

Claim 35 is directed to a method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order. Like claim 2 discussed above, claim 35 has at least the following attributes not disclosed by the references cited: for example, (a) the use of *at least three different DNA vectors, each comprising a DNA segment and a selectable marker*; (b) the first DNA vector is cleaved so that *the resulting linear DNA molecule comprises a DNA segment that is covalently associated with first vector sequences comprising sequence encoding the first selectable marker*, and *the second DNA vector is cleaved so that the resulting linear DNA molecule comprises a DNA segment that is covalently associated with second vector sequences comprising sequence encoding the second selectable marker*; (c) simultaneously ligating each segment to the adjacent segment or segments and ligating the first vector sequence to the second vector sequence, wherein *said DNA segments from said at least three different DNA vectors are not purified prior to said ligating, thereby producing a closed circular DNA molecule comprising said segments, said first selectable marker, and said second selectable marker*; (d) selecting a ligation product comprising sequences from each of said DNA segments in a predetermined order, wherein *said selection is based on the presence in the ligation product of the first selectable marker, the second selectable marker, or both the first and second selectable markers*. As discussed above, these unique attributes should overcome the references cited by the Examiner.

Therefore, Applicants respectfully request that the rejection of claim 35 under 35 U.S.C. § 103(a) be withdrawn.

V. Claims 3-13, 15-19, 31-34, and 36-39

Claims 3-13 are directed to a method for ligation of a plurality of DNA segments, directly or indirectly dependent from claim 2; claims 15-19 are directed to compositions of an unpurified

cleavage products of DNA molecules and a DNA ligase, directly or indirectly dependent from claim 14; claims 31-34 are directed to a method of ligation of a plurality of DNA segments, directly or indirectly dependent from claim 1; and claims 36-39 are directed to a method for ligation of a plurality of DNA segments, directly or indirectly dependent from claim 35. The Examiner rejected these claims in sweeping language without elaborating how all of the limitations of these claims are taught by the references cited.

"If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious." (MPEP § 2143.03). As discussed above, the references cited by the Examiner, when combined, do not render independent claim 1, 2, 14, or 35 obvious; therefore, neither are dependent claims 3-13, 15-19, 31-34, and 36-39 obvious over Hodgson, in view of Padgett et al., Resnick et al., certain "Stewart et al.," and Gokhale et al.

Moreover, the references cited do not teach, but not limited to, the following features of the present invention:

(1) in claim 3: "selecting transformants that express said first and second selectable markers and do not express said first, second, or third counter-selectable marker";

(2) in claim 5: "said first and second selectable markers are genes conferring drug resistance";

(3) in claim 6: "said first, second and third counter-selectable markers are selected from the group consisting of ccB (anti-DNA gyrase protein), sacB (sucrose sensitivity), araB (ribulose sensitivity), and tetAR (tetracycline resistance/fusaric acid hypersensitivity";

(4) in claim 7: "a) said first and third cleavage sites are the same; b) said second and fourth cleavage sites are the same; c) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 3-prime cleavage site of the same Type 3 DNA molecule; and/or d) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 5-prime cleavage site of a different Type 3 DNA molecule";

(5) in claim 11: "the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polypeptide segment of a polyketide synthase";

(6) in claim 12: "the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polyketide synthase domain";

(7) in claim 13: "the DNA molecules cleaved in step (b) are cleaved in the same container";

(8) in claim 15: The composition of claim 14 "comprising at least two Type 3 DNA molecules";

(9) in claim 16: The composition of claim 14 "comprising an endonuclease that cleaves at the first, second, third, or fourth cleavage sites or at one or more 5-prime or 3-prime cleavage sites";

(10) in claim 18: The composition of claim 16 that "contains at least two Type 3 DNA molecules comprising 5-prime or 3-prime cleavage sites and wherein the endonuclease cleaves at the third and fourth cleavage sites and at the 5-prime and 3-prime cleavage sites of said Type 3 DNA molecules";

(11) in claim 19: The composition of claim 14, wherein, "after a sufficient amount of time for a ligation reaction using said DNA ligase, a product is formed, the product comprising: the first DNA segment immediately upstream of the second DNA segment, wherein the second DNA segment is immediately upstream of the DNA segment from at least one Type 3 DNA molecule; and at least one selectable marker";

(12) in claim 31: "the selectable markers in (a) are sequences encoding a protein that confers drug resistance to a host, and the selection in step (d) is based on the presence in a vector comprising the ligation product of two different selectable markers, wherein the two different selectable markers are associated with two different vectors in step (a)";

(13) in claim 32: "the selectable marker provides resistance to a drug selected from the group consisting of carbenicillin, tetracycline, kanamycin, streptomycin, and chloramphenicol";

(14) in claim 33: "the selection in step (d) is based on the absence in a vector comprising the ligation product of a counter-selectable marker present in one or more of the vectors in (a)";

(15) in claim 36: "said selecting is based on the presence in the ligation product of both the first and second selectable markers";

(16) in claim 37: "the selectable markers provide resistance to a drug selected from the group consisting of carbenicillin, tetracycline, kanamycin, streptomycin, and chloramphenicol"; and

(17) in claim 39: "at least one of the selectable markers is a counter selectable marker selected from the group consisting of *ccdB* (anti-DNA gyrase protein), *sacB* (sucrose sensitivity), *araB* (ribulose sensitivity), and *tetAR* (tetracycline resistance/fusaric acid hypersensitivity)."

Therefore, Applicants respectfully request that rejections of dependent claims 3-13, 15-19, 31-34, and 36-39 be withdrawn.

Summary

To support a rejection based on obviousness, the Examiner must address all claim limitations, and must explain with specificity why one of ordinary skill in the art would have been motivated to carry out the claimed method. The references cited by the Examiner neither described nor suggested Applicants' method invention.

More particularly, the use of restriction sites and selectable and counter selectable markers to build synthetic genes as claimed and described in the specification is highly innovative and allows, *inter alia*, rapid and economical gene synthesis without the intervening isolation of fragments as required by methods such as those disclosed in Hodgson. Padgett et al. does not teach "ligating the desired DNA segments without purifying the digested fragments" either explicitly or implicitly. Resnick et al. was made in a different context to serve different purposes and does not teach use of a selectable marker and/or counter selectable marker as used in the present invention. Gokhale et al. does not have any bearing on the independent claims 1, 2, 14, and 35, and if at all, only bears a tenuous relationship with the dependent claims 11 and 12, which is short of motivating a person of ordinary skill in the art to pursue a method as claimed in this application for preparing synthetic genes of PKS.

Therefore, Applicants respectfully request that rejections of claims 1-19 and 31-39 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

In view of these amendments and remarks, Applicants believe that the claims of this application are in condition for allowance and an early notice to this effect is earnestly solicited. If the Examiner does not believe that such action can be taken at this time or if the Examiner feels that a telephone interview is necessary or desirable, Applicants welcome the Examiner to call the undersigned at 609-844-3020.

No fees are believed to be due upon filing of this paper. To the extent that any fees are required, in connection with receipt, acceptance and/or consideration of this paper and/or any accompanying papers submitted herewith, please charge all such fees to Deposit Account 50-1943.

Respectfully submitted,

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By: /Gerard P. Norton/

Gerard P. Norton, Reg. No. 36,621
Fox Rothschild LLP
Princeton Pike Corporate Center
997 Lenox Drive, Building 3
Lawrenceville, NJ 08648-2311
Telephone: (609) 844-3020
Facsimile: (609) 896-1469
Attorney for the Applicant(s)